In the Specification:

On page 1, line 5, please replace the present paragraph with the following paragraph:

KK 10/15/03

B1

This patent application claims priority to U.S. patent application Serial No. 09/699,136, filed 27 October 2000, which claims priority to U.S. Provisional Application Serial No. 60/161,703, filed 27 Oct. 1999, and is related to Serial Nos. 60/161,414, filed 25 Oct. 1999, and 60/206,082, filed 18 May 2000, each of which is incorporated herein by reference.

On page 6, line 17, please replace the present paragraph with the following paragraph:

B2

--The present invention provides recombinant host cells and expression vectors for making products in host cells, which are otherwise unable to make those products due to the lack of a biosynthetic pathway to produce a precursor required for biosynthesis of the product. As used herein, the term recombinant refers to a cell, compound, or composition produced at least in part by human intervention, particularly by modification of the genetic material of a cell. The present invention also provides methods for increasing the amounts of a product produced in a host cell by providing recombinant biosynthetic pathways for production of a precursor utilized in the biosynthesis of a product.--

On page 12, line 25, please replace the present paragraph with the following paragraph:

B3

Thus, in accordance with the methods of the invention, the heterologous production of certain polyketides in *E. coli,* yeast, and other host organisms require both the heterologous expression of a desired PKS and also the enzymes that produce at least some of the substrate molecules required by the PKS. These substrate molecules, called precursors, are not normally found as intracellular metabolites in the host organism or are present in low abundance. The present invention provides a method to produce or modify the composition or quantities of intracellular metabolites within a host organism where such metabolites are not naturally present or are present in non-optimal amounts.

At page 13, line 22, please replace the present paragraph with the following paragraph:

The MUT pathway includes the enzymes methylmalonyl CoA mutase (5.4.99.2, using the numbering system devised by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology), methylmalonyl CoA epimerase (5.1.99.1), and malonyl CoA decarboxylase (4.1.1.9). The biochemical pathway includes the conversion of succinyl CoA to (R)-methylmalonyl CoA through the action of methylmalonyl CoA mutase (5.4.99.2) followed by the conversion of (R)-methylmalonyl CoA to (S)-methylmalonyl CoA through the action of methylmalonyl CoA epimerase (5.1.99.1). (S)-methylmalonyl CoA is a substrate utilized by several polyketide synthases. The enzyme malonyl CoA decarboxylase (4.1.1.9) catalyzes the decarboxylation of malonyl CoA but is also reported to catalyze the decarboxylation of (R)-methylmalonyl CoA to form propionyl CoA. Propionyl CoA is a substrate utilized by some polyketide synthases.

B4

At page 14, line 13, please replace the present paragraph with the following paragraph:

An illustrative embodiment of the present invention employs specific enzymes from these pathways. As those skilled in the art will recognize upon contemplation of this description of the invention, the invention can also be practiced using additional and/or alternative enzymes involved in the MUT and PCC pathways. Moreover, the invention can be practiced using additional and alternative pathways for methylmalonyl CoA and other intracellular metabolites.

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At page 19, line 28, please replace the present paragraph with the following paragraph:

A suitable propionyl CoA carboxylase (6.4.1.3) gene for purposes of the present invention can be isolated from *Streptomyces coelicolor* as reported in GenBank locus AF113605 (pccB), AF113604 (accA2) and AF113603 (accA1) by H. C. Gramajo and colleagues. The propionyl CoA carboxylase gene product requires biotin for activity. If the host cell does not make biotin, then the genes for biotin transport can be transferred to the host cell. Even if the host cell makes or transports biotin, the endogenous biotin transferase enzyme may not have sufficient activity (whether due to specificity constraints or other reasons) to biotinylate the propionyl CoA carboxylase at the rate required for high level precursor synthesis. In this event,

B6

one can simply provide the host cell with a sufficiently active biotin transferase enzyme gene, or if there is an endongenous transferase gene, such as the *birA* gene in *E. coli*, one can simply overexpress that gene by recombinant methods. Many additional genes coding for propionyl CoA carboxylases, or acetyl CoA carboxylases with relaxed substrate specificity that includes propionate, have been reported and can be used as sources for this gene, as shown in the following table.

At page 34, line 9, please replace the present paragraph with the following paragraph:

Methods for generating libraries of polyketides have been greatly improved by cloning PKS genes as a set of three or more mutually selectable plasmids, each carrying a different wild-type or mutant PKS gene, then introducing all possible combinations of the plasmids with wild-type, mutant, and hybrid PKS coding sequences into the same host (see U.S. ~~patent~~ provisional application Serial No. 60/129,731, filed 16 Apr. 1999, and PCT Pub. No. 98/27203, each of which is incorporated herein by reference). This method can also incorporate the use of a KS1° mutant, which by mutational biosynthesis can produce polyketides made from diketide starter units (see Jacobsen *et al.*, 1997, *Science* **277**, 367-369, incorporated herein by reference), as well as the use of a truncated gene that leads to 12-membered macrolides or an elongated gene that leads to 16-membered ketolides. Moreover, by utilizing in addition one or more vectors that encode glycosyl biosynthesis and transfer genes, such as those of the present invention for megosamine, desosamine, oleandrose, cladinose, and/or mycarose (in any combination), a large collection of glycosylated polyketides can be prepared.

At page 56, line 15, please replace the present paragraph with the following paragraph:

To express active mutase *in vivo*, it was necessary to grow cells in a defined media (MUT media) that allows uptake of the vitamin B12 precursor hydroxocobalamin; this is similar to an established protocol for expression of active methionine synthase, which also requires B12. Cell extracts overexpressing the mutase were shown to convert mm-CoA to succinyl CoA without the addition of vitamin B12. Only one time point (at 20 minutes) was assayed to confirm activity; the specific activity of the mutase was not determined.

At page 56, line 22, please replace the present paragraph with the following paragraph:

Thus, methylmalonyl-CoA mutase was expressed as the active holoenzyme in *E. coli*, and methylmalonyl-CoA was produced in vivo. Because a slow, spontaneous chemical epimerization between (R)- and (S)-mm-CoA does exist (approximately 3% in 15 minutes), it may be helpful to determine the relative amounts of these diastereomers in cells overexpressing the mutase. Enough (S)-mm-CoA may be present to support polyketide production in some cells without addition of an epimerase. To facilitate the eventual production of polyketides in *E. coli*, the mutase gene can be incorporated into the chromosome of the BL21 *panD* cell or other host cell.

At page 66, line 28, please replace the present paragraph with the following paragraph:

The sequence of the putative epimerase gene contained in cosmid 117-167-A7 was aligned to the N-terminal epimerase sequence already known. The several hundred base pairs downstream of this sequence were translated in all three frames and a stop codon in one of the frames was found that yielded a protein of the expected size. The entire sequence was used to search the protein database via BLAST analysis, and the sequence showed high homology to the sequence of a putative epimerase from *S. coelicolor* identified in accordance with the methods of the invention. PCR primers were designed based on the DNA sequence of the cloned P. shermanii epimerase and the gene was amplified from *P. shermanii* genomic DNA with *Nde*I and *Bam*HI sites at the 5'-end, an internal *Nde*I site was destroyed near the 5' end, and *Nhe*I and *Avr*II sites were introduced at the 3'-end. Following PCR, the 447 bp product was cloned into a Bluescript vector (143-6-11) and sequenced. Also, four additional sequencing primers were designed to provide several-fold coverage of the epimerase gene. The full epimerase gene sequence (SEQ ID NO:1) and encoded amino acid sequence (SEQ ID NO:2) provided in isolated and recombinant form by the present invention is shown below.

At page 69, line 26, please replace the present paragraph with the following paragraph:

Epimerase activity was measured in crude extracts of *E. coli* harboring either pET-epsherm, pET-epcoel, pET-epsub, or a control pET vector. The epimerase assay couples

transcarboxylase, which converts (S)-methylmalonyl-CoA into propionyl-CoA, to malate dehydrogenase, which converts NADH into NAD^+, producing a decrease in absorbance at 340 nm. The assay is initiated with a racemic mixture of (R,S)-methylmalonyl-CoA; when the (S)-isomer is consumed as described below, a steady background rate is observed at about one-tenth of the initial rate. When an extract containing epimerase is added to the assay, the (R)-isomer is converted to (S)-, resulting in a further decrease in absorbance. In crude *E. coli* extracts, however, a significant background rate is observed, probably due to an endogenous NADH oxidase. Thus the epimerase must be expressed at a sufficiently high level to conclude that it is active. The assay was conducted as follows.

At page 70, line 10, please replace the present paragraph with the following paragraph:

The pellet from approximately 20 mL of culture was thawed and resuspended in 2 mL 1X assay buffer containing a protease inhibitor cocktail tablet. The cells were disrupted by sonication (two sonication cycles for 30 seconds each at a power setting of 2 [pulse ON 0.5 sec/pulse OFF 0.5 sec]). After spinning for 10 minutes at 13,000 rpm in an Eppendorf centrifuge, the supernatants were saved for assay. Methylmalonyl-CoA epimerase activity was assayed using a modification of the method of Leadlay *et al.* (1981). The assays were performed at 30°C with a 1 cm path length plastic cuvette, in a final volume of 1.5 mL. The reaction mixtures contained 0.2 M potassium phosphate buffer pH 6.9, 0.1 M ammonium sulfate, 5 mM sodium pyruvate, 0.08 mM (2*R*,2*S*)-methylmalonyl-CoA, 0.05 units of partially purified transcarboxylase, 0.16 mM NADH, and 2.5 units malate dehydrogenase. The reaction was initiated with (2*R*,2*S*)-methylmalonyl-CoA and the decrease in absorbance at 340 nm was monitored, reflecting the disappearance of the 2*S* isomer. When the decrease in absorbance at 340 nm reached the basal level (usually around 10% of the initial transcarboxylase rate), an extract containing epimerase was added and a further decrease in absorbance was observed. The chemicals and enzymes used in the epimerase assay were purchased from Sigma, except for transcarboxylase, which was obtained as a crude preparation from Case Western Reserve.

At page 73, line 28, please replace the present paragraph with the following paragraph:

Most modular PKSs require either or both malonyl-CoA or (2*S*)-methylmalonyl-CoA as a source of 2-carbon units for polyketide biosynthesis. The malonyl-CoA pools in yeast are quite sufficient for polyketide synthesis, as illustrated by the production of large amounts of 6-MSA in yeast. However, *S. cerevisiae* does not produce (2*S*)-methylmalonyl-CoA and does not possess biosynthetic pathways for methylmalonyl-CoA biosynthesis. Hence, a heterologous biosynthetic pathway must be introduced into *S. cerevisiae* to support biosynthesis of polyketides that use (2*S*)-methylmalonyl-CoA as a precursor.

On page 82 line 11, please replace the present paragraph with the following paragraph:

As described in Example 1, methylmalonyl-CoA epimerase was purified from *Propionibacterium shermanii* and N-terminal and internal protein sequence was obtained. Degenerate PCR primers based on the amino acid sequences were designed and were used to amplify a 180 bp PCR product from *P. shermanii* genomic DNA. The PCR product was labeled and used to isolate the epimerase gene from *P. shermanii*. The methylmalonyl-CoA epimerase genes from *B. subtilis* [16] and *S. coelicolor* can also be employed in the methods of the present invention.

At page 83, line 1, please replace the present paragraph with the following paragraph:

Propionyl-CoA is not detected in *E. coli* SJ16 cells grown in the presence of [^{3}H] ß-alanine with or without the addition of propionate in the growth media. When *E. coli* SJ16 cells were transformed with a pACYC-derived plasmid containing the *Salmonella typhimurium* propionyl-CoA ligase gene (*prpE)* under the control of the *lac* promoter, a small amount of propionyl-CoA was observed (~0.2% of total CoA pool) in cell extracts. When 5 mM sodium propionate was included in the culture medium, about 14-fold more propionyl-CoA was produced (~ 3% of the total CoA pool).